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Effect of Casing Layer on Growth Promotion of the Edible Mushroom *Pleurotus ostreatus*

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Various bacteria were isolated from the casing layer soil of the culture bed of *P. ostreatus* and their role in fruiting body induction of the edible mushroom, *P. ostreatus*, was investigated. Analysis of the bacterial community isolated from the casing layer soil revealed that the composition of genera and number of cultivable bacteria were different for each sterilizing treatment. *Bordetella* was predominant in the bulk soil whereas *Flavobacterium* was predominant after sterilization of the casing layer soil. Fluorescent *Pseudomonas* was predominant in the non-sterilized casing layer soil. Total number of the bacterial genera in the casing layer soil was higher than that in the bulk soil. In particular, an increase in the fluorescent *Pseudomonas* population was observed in the non-sterilized casing layer accompanied by induction of fruiting body and enhanced mushroom production yield. The results suggested that specific bacterial populations in the casing layer play an important role in the formation of primodia and the development of basidiome in *P. ostreatus*.

KEYWORDS : Casing layer soil, Mushroom growth promotion, Mushroom production, *Pleurotus ostreatus*

The oyster mushroom, *Pleurotus ostreatus*, is one of the most widely cultivated mushrooms in the world (Baars *et al.*, 2000). Mushrooms are economically important biotechnological products and their market has markedly expanded across the world in the past few decades. Mushrooms, the fruiting bodies of basidomycetes, have been traditionally consumed as a food and also have been considered as some of the potential nutraceuticals (Chang *et al*., 1996; Kues *et al*., 2000). The oyster mushroom is a popular cultivated mushroom along with the white button mushroom, *Agaricus bisporus*. Techniques for its cultivation are similar to those used for *A. bisporus* that the fungi are grown on various organic composts. Many microorganisms are known to promote the growth of *P. ostreatus* and other cultivated mushrooms (Cho *et al*., 2003). The microorganisms found in the casing layers are especially important for induction of fruiting body formation (Eger, 1961, 1972; Grewal and Rainey, 1992; Rainey *et al*., 1990). Particluarly, the growth and development of *A. bisporus* are known to be affected by fluorescent *Pseudomonas* (Cho *et al*., 2003; Grewal and Rainey, 1992). To date, detailed studies on the interaction between edible fungi and bacteria have been primarily conducted with strains of *Pseudomonas putida*, which initiate basidiome formation and stimulate fungal growth. Recent work has revealed that the interaction is closely related to the rate of hyphae extension and the formation of primodia (Rainey, 1991). On the contrary, the roles of the fluorescent *Pseudomonas* in the cultivation of *P. ostreatus* are less understood. The current method of forcing mushroom mycelia to change from the vegetative to the reproductive phase involves the application of a covering of some suitable materials (the casing layer) on the surface of the spawn compost (Hume and Hyes, 1972; Mac Canna and Flanagan, 1972; Peerally, 1979). The casing also functions to supply and conserve moisture for the mushrooms and their rhizomorphs as well as to act as the transport system for dissolved nutrients.

There can be a wide variation in the yields of mushrooms depending on different casing layer materials, leading to the question of the affect of the biological properties of the various casing layers. Based on recent studies involving *A. bisporus*, there is also considerable interest in the use of bio-augmentation with mushroom-promoting *Pseudomonas* to improve the consistency of different casing layers for production. Accordingly, the primary purpose of this study was to compare the population of microorganisms and mushroom-production in sterilized casing layers with those in non-sterilized casing layers.

Materials and Methods

Conditions of the casing layer soil. Soil for the casing layer was collected from a commercial *A. bisporus* company located in Buyeo-gun, Choongchungnam-do. The soil was treated in one of two ways. One case included a sterilized casing layer, while the other included a layer that was not sterilized. These two types of conventional

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casing layer soils and their initial ingredients were obtained from a commercial farm, Samgu, in Yeoju, Korea. Phase 1 is a non-sterilized casing layer soil still containing mycelium. Phase 2 is a sterilized casing layer soil obtained by sterilizing the sample at 121°C for 90 min and repeating after cooling at room temperature. The sample soil was maintained in its original condition below the casing layer. The amount of casing layer soil covering the mycelium of *P. ostreatus* was fixed at 2 kg and the casing layer was evenly spread. We investigated only the population of microorganisms for this study.

Cultivating conditions for mushrooms. *P. ostreatus* was cultured with cotton plant waste products in a culture room. Underground water was used to adjust the moisture of the cotton plant waste products. The cotton waste put in a 40 × 40 × 15 (cm) box to heat at 65°C for 14 h. When the temperature of the cotton plant waste decreased to 25°C, we inoculated the cotton plant waste with spawn of *P. ostreatus*. The cultivation temperature was maintained at 24°C for 17 days in a mycelium growth chamber. When the spawn became sufficient maturity, the casing layer was applied on the mycelium, those was cultured and the reproduction growth was induced at 18°C.

Distribution of the microbial populations. *P. ostreatus* was cultured on potato dextrose agar (PDA). Bacteria

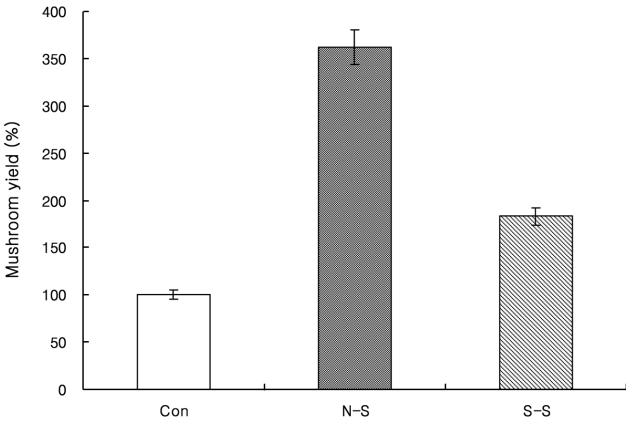

Fig. 1. Comparison of yield patterns of *Pleurotus ostreatus* by casing layer treatment. □, control:general cultivated medium; ■, N-S: Yield from non-sterilized casing layer soil; ■, S-S: Yield from sterilized casing soil.

from the casing layer of *P. ostreatus* were cultured on 10% TSA and P1 medium (Cho *et al*., 2003). Actinomycetes were grown on humic acid vitamin (HV) agar, and fungi were grown on Rose-Bengal agar. For identification of bacteria by fatty acid analysis, bacteria isolated from the casing layer soil of *P. ostreatus* were grown on TSA. Bacteria isolated from the casing layer soils were identified by fatty acid methyl ester (FAME) analysis following cultivation of the individual isolates on TSBA for

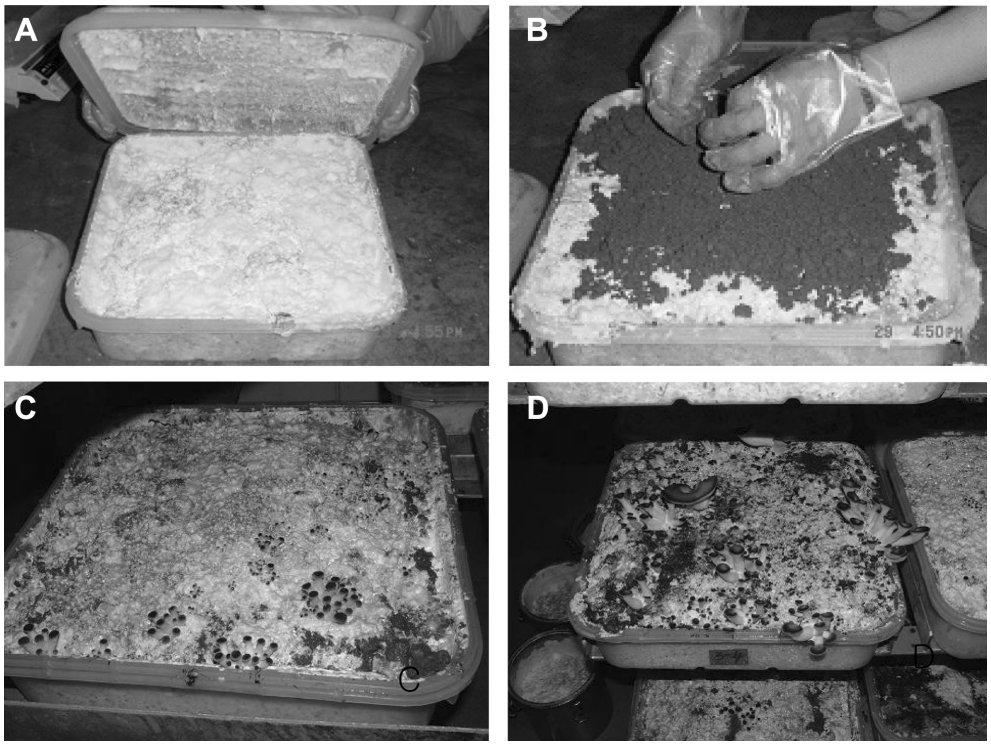

Fig. 2. Casing layer treatment process and growth of *P. ostreatus*. A: Completed nutritional growth of mycelium. B: Casing layer on mycelium. C: Initiated primodia formation on the casing layer. D: Promoted fruiting body.

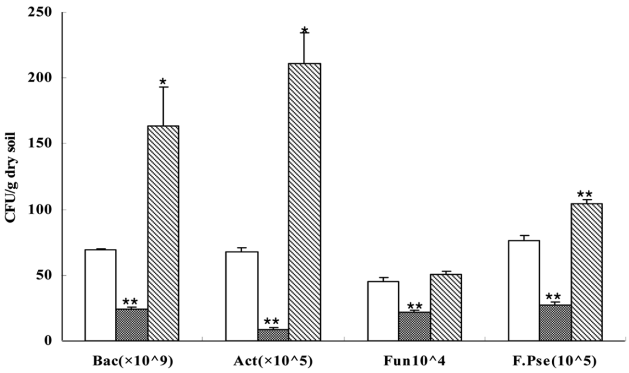

Fig. 3. Distribution of the microbial population of the casing layer treatment. Bac, aerobic bacteria; Act, actinomycetes; Fun, fungi; F. Pse, fluorescent *Pseudomonas*. Vertical bars indicate mean standard error. □: Bulk soil, ▒: S-S soil: Sterilized casing layer soil, ▨: N-S soil: Non-sterilized casing layer soil. Data are shown as the Mean ± SE. $*P > 0.05$, $**P > 0.01$.

24 h at 28°C prior to the analysis. FAME(fatty acid methyl ester) was conducted using the Sherlock Microbial Identification System (MIDI, Inc., Newark, DE, USA), using protocols recommended by the manufacturer. Bacteria were collected from the casing layer soil (sterilized and non-sterilized) after *P. ostreatus* was harvested (Fig. 2). The casing layer soil for this experiment was chosen according to the highest yield from 6 boxes of planted mushrooms which divided into sterilized soil (S-S), non-sterilized (N-S) soil and bulk soil.

Statistics. All experiments were performed in triplicate. Statistical significance was determined by Student's *t*-test, and data were expressed using mean standard error (SE). Total numbers of types of fungi, bacteria, actinomycetes and fluorescent *Pseudomonas* in the casing layer soils and bulk soil are shown in Fig. 3.

Results

Effect of casing layer on the growth promotion of *P. ostreatus*. Growth of *P. ostreatus* was investigated according to casing layer treatment. The harvest yield and production frequency from the first harvesting period to 312 hours after the first harvest are reported here. In the harvest yield, 950 grams of mushrooms were produced in the control while 1,501 grams were produced in the S-S soil. The N-S sample produced a total of 2,967 grams, considerably more than S-S. Also, the frequency of fruiting body formation was higher in N-S than in S-S: 4 positions in control, 6 positions in S-S and 13 positions in N-S.

Table 1. Major bacterial distribution among isolates from casing layer soil samples

Isolated bacterial	Phyla	Bulk soil	Casing soil treatment	
			Sterilization (S-S)	Non-Sterilization (N-S)
Acidovorax	Beta-proteobacteria		3	1
Actinomadura	Actinobacteria		1	
Agrobacterium	Alpha-proteobacteria			1
Alcaligenes	Beta-proteobacteria		2	1
Aquaspirillum	Beta-proteobacteria		3	2
Arthrobacter	High G+C			2
Bacillus	Low G+C	1	1	1
Bordetella	Beta-proteobacteria	16	1	
Brevundimonas	Alpha-proteobacteria		3	3
Cellulomonas	High G+C			1
Deinococcus	Deinococcus-Thermus			
Erwinia	Gamma-proteobacteria	2	1	
Flavobacterium	CFB		10	6
Micrococcus	Actinobacteria			1
Micromonospora	Actinobacteria			1
Paenibacillus	Low G+C	3		1
Pedobacter	Bacteroidetes	3	1	2
Photobacterium	Gamma-proteobacteria			1
Pseudomonas	Gamma-proteobacteria	9	6	11
Ralstonia	Beta-proteobacteria	1	2	1
Sphingobacterium	Bacteroidetes		7	6
Streptoverticillium	Actinobacteria	2		
Variovorax	Beta-proteobacteria		1	
Yersinia	Gamma-proteobacteria		3	2
No match		10	14	14
Total		47	59	56

Analysis of bacterial populations in the casing layer of *Pleurotus ostreatus*. Total culturable bacteria were enumerated in the casing layer soil chosen from each of 6 boxes of mushroom samples, according to highest yield. The population size of the total cultivable microorganisms was significantly higher ($P < 0.05$) in the high yielding non-sterilized growth medium than in the high yielding sterilized growth medium (Fig. 3). The fungal, actinomycetes, bacterial and fluorescent *Pseudomonas* population densities were higher in the N-S soil than in the S-S soil.

The casing layer from N-S medium with the high-yield had approximately 3.3×10^9 CFU g^{-1}, whereas the bacteria from S-S medium with the high-yield had 9.8×10^8 CFU g^{-1}.

Analysis of the aerobic bacteria community in a casing layer soil. A total of 162 colonies of bacteria from the bulk soil and the two casing layer samples were isolated. These results are shown in Table 1. The bacteria were: 8 genera in the bulk soil, 15 genera in the sterilized treatment of casing layer samples and 18 genera in the non-sterilized treatment casing layer samples, as determined by FAME. Altogether, 41 genera of bacteria were identified (Table 1). Regarding the relationship of soil bacterial community composition, the bulk soil had a high frequency of *Bordetella* and *Pseudomonas*. The sterilized treatment of casing layer samples had a high frequency level of *Flavobacterium* and *Sphingobacterium*. In contrast, the non-sterilized treatment of casing layer samples showed various clustering patterns of the bacteria *Pseudomonas*, *Flavobacterium* and *Sphingobacterium* (Table 1).

Discussion

P. ostreatus is the most widely cultivated mushroom in the world, followed by *A. bisporus*, and the demand for these edible mushrooms is increasing. Cultivation of *P. ostreatus* is based primarily on the techniques used for cultivating *A. bisporus*. Similarities in cultivation include a pre-treatment process of the growth medium through a casing layer soil. The study of this fruiting mechanism is especially so important that its potential possibly improve the output of industrially cultivated species. Given proper environmental conditions such as nutrition, light and temperature, the mushroom eventually develops a multi-cellular structure, also known as the fruiting body world (Baars *et al.*, 2000). The biological mechanism of fruiting body formation has been reported previously by many sources (Caselton and Olesnicky, 1998; Kues, 2000; Kues and Liu, 2000). For example, a cover of a suitable material (the casing layer) on the surface of the spawn compost is required to change *A. bisporus* mycelia from the vegetative phase to a reproductive state (Eger, 1972; Hayes 1984; Peerally, 1979). The results of this study (Fig. 1) showed the casing layer treatment process and the subsequent growth of *P. ostreatus* as well as *A. bisporus*. *P. ostreatus* samples were cultivated on 2 types of soil, including the control. Mushroom yields were as follows: 950 grams from the control, 1,501 grams from the sterilized casing layer and 2,967 grams from the non-sterilized casing layer. Interestingly, the time of fruiting body initiation was approximately 6 days earlier with a non-sterilized casing layer than with a sterilized casing layer, and 9 days earlier than the control (data not shown). The purpose of this experiment was to determine whether soil microorganisms were promoted with a casing layer. According to the results of this study, as modified from the methods of Eger (1972), mycelium of *P. ostreatus* grew well with the presence of a casing layer, and the formation period of fruit body was decreased. The population of the bacteria was higher in N-S than in the S-S, as was the fungal, fluorescent *Pseudomonas* and actinomycetes population densities. These results correspond with those of Rainly (1991). The density of *Pseudomonas* was high in S-S, and especially high in N-S. Since it has been suggested that fluorescent *Pseudomonas* promotes growth of *P. ostreatus*, this density may also have a close relationship with the formation of fruiting body. While the bulk soil had a high frequency of Beta and Gamma-proteobacteria, the sterilized treatment (S-S) showed CFB group bacteria and Beta-proteobacteria were predominant, similarly to the non-sterilized treatment: CFB group bacteria and Gamma-proteobacteria (Table 1). Accordingly, the casing layer soil may be considered a good material for promotion of the growth of *P. ostreatus*, as well as composting, which is very important for increasing yields of both *P. ostreatus* and *A. bisporus*. Recently, the harvesting ratio of *P. ostreatus* is smaller than *A. bisporus*, due to the omission of casing layers and composting processes. Also, many of the *P. ostreatus* cultivators in Korea are not aware of the role of microorganisms in the growth of *P. ostreatus*. In the future, the harvest yield of *P. ostreatus* may be increased if additional studies regarding beneficial microorganisms are performed, and interest in these areas expanded.

Acknowledgments

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